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Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on the HPV Test Plan for the Ethylphenols Category

Dear Administrator Whitman:

The following comments on the Merisol USA LLC High Production Volume (HPV) Challenge test plan for the chemical class known as ethylphenol isomers are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

Merisol submitted its test plan on July 29, 2002. The ethylphenols category is comprised of three ethylphenol isomers, as follows:

Chemical	CAS Number
o-ethylphenol	9006
p-ethylphenol	123079
m-ethylphenol	620177

Merisol states in its test plan that, since the ethylphenols are generally moved into commerce as starting materials for further chemical processing, there is little consumer exposure. However, due to the large production volume of these chemicals, they are subject to the HPV program. Most are sold by Merisol as blends and not as isolated isomers.

Overall, the test plan for mixed ethylphenols proposes limits on the amount of new animal testing by grouping the various isomers of ethylphenols into one testing category. While we agree with this approach, which results in fewer animals being used in the SIDS battery, we remain concerned about the proposed testing, which includes the following:

1. Acute oral toxicity study (OECD No. 425),
2. Combined repeat dose/reproductive/developmental study (OECD No. 422) and
3. Mammalian erythrocyte micronucleus test (OECD No. 474).

All of these tests are unnecessary. If this test plan is conducted in its present form, approximately 810 animals will be killed. In addition, an acute fish toxicity study (OECD No. 203) is proposed which is also unwarranted. This study would require an additional 40 animals. Our objections are summarized later in these comments.

Merisol bases the chemical category of the ethylphenols on their chemical similarity, i.e., they are all phenols substituted with one ethyl group in one of the three positions on the phenolic ring (i.e., ortho, meta, and para positions), sharing the same molecular weight, or, in the case of the mixture, average molecular weight, and the physical-chemical properties of the isomers are similar. In addition, as stated in the Merisol test plan, methyl phenols, known as cresols, "have demonstrated that the methyl phenol isomers are remarkably equivalent in toxicity and that binary and tertitiary mixtures of cresol isomers do not produce toxic interactions among the isomers, i.e., mixtures of cresol isomers do not exhibit more than additive toxicity." In addition, the data on the cresols demonstrated "that there was no one predominately toxic isomer and that target organ for toxicity and toxic dose levels were relatively consistent across the isomers." A similar pattern would be expected for the ethyl phenols based on structural similarity among this group of isomers. Again, we agree with this assessment.

However, on page 6 of the Merisol test plan, the company states that the "details for the toxicological work on ethylphenols are unavailable….[and] no ***existing studies*** will be relied upon for HPV evaluations. Accordingly, Merisol proposed that no ***existing studies*** will be used to supply data for SIDS endpoints in the Ethylphenols Category. Merisol is not relying on data developed on analogous compounds to satisfy Ethylphenols Category but instead will develop data for each SIDS Screening Endpoint using the ethylphenol isomer mixture…. Merisol is defining ethylphenols [to be tested] as a mixture containing equal portions of o-, p- and m-ethylphenol." (emphasis added).

We strenuously object to this approach, with its inexplicable and indefensible disregard of existing data, as it will result in the needless testing and suffering of animals and violates principles of thoughtful toxicology and good science. Our objections are summarized below:

1. Merisol does not follow some of the main tenets laid out in the *Federal Register* notice in December 2000 regarding the development of test plans. It is not appropriate to create a testing category, reject the use of toxicity data on individual components in that same category, and then conduct new animal tests on an arbitrary mixture of the individual components. Apparently there are data available on the individual isomers, as Merisol states on page 6 "…that no ***existing studies*** will be used to supply data for SIDS endpoints in the Ethylphenols Category" (emphasis added). The test plan appears to deliberately ignore information available to Merisol on the individual isomers of ethylphenol that can reduce the use of animals in SIDS tests. The EPA has clearly stated that

all available data should be carefully considered before new animal tests are conducted. By choosing to ignore available data and not summarizing these data in its test plan, Merisol has violated this basic principle. We request in the strongest terms that any data available to Merisol on the isomers be summarized and its use maximized to reduce the amount of new animal tests on the mixture. In addition, any new data (from new animal tests) should be relevant in terms of hazard assessment and not duplicative of any ***existing data*** on the individual isomers. This is also a stated goal in the Federal Register, but this cannot be determined if existing data have been ignored and not included in the test plan (emphasis added). Perhaps Merisol is unaware of the guidance the EPA has provided to manufacturers in the development of test plans and the goals of minimizing the use of animals in the HPV program.

2. We have identified other data which have not been included in the evaluation of the ethylphenols and may be useful in determining data gaps. As noted above, an evaluation of all relevant information is required in the December 2000 *Federal Register*. The citations for this information are provided below, specifically:

   Initial Submission: Toxicity Report: M-Ethylphenol With Cover Letter Dated 09/28/92 (1992; EPA/OTS; Doc. #88-920009161).

   Initial Submission: Acute and Irritation Studies With 4-Ethylphenol in Rats and Rabbits With Cover Letter (1992; EPA/OTS; Doc. #88-920004538).

   Ambient Working Water Quality Guidelines for Phenols: Technical Report (April 19, 2002; prepared by the Water, Air and Climate Branch, Ministry of Water, Land and Air Protection, British Columbia, Canada) (Contains LC_{50}'s for 4-ethylphenol in fathead minnow plus extensive analysis of aquatic toxicity of various phenols in many different test systems). (http://wlapwww.gov.bc.ca/wat/wq/Bcguidelines/phenol/phenol.html)

   Safety data for o-ethylphenol (an MSDS showing two LD_{50}'s in mice of 600 mg/kg [oral] and 172 mg/kg [intraperitoneal]) (http://www.physchem.ox.ac.uk/MSDS/ET/o-ethylphenol.html)

   MSDS (Aldrich Chemical Company, valid through 1/2002, showing an interperitonel LD_{50} in mice of 138 mg/kg) (http://www.conncoll.edu/offices/envhealth/MSDS/chsmistry/E/4-Ethylphenol,-99.html).

   Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals (1992; Zeiger et al.; Environmental Molecular Mutagen, 19 Suppl21:2-141).

A thorough evaluation of all of the toxicity data as a whole would likely obviate the need for additional animal testing under the HPV program. We strongly request that Merisol access this information and revise its testing plan accordingly. This will assist in further reducing the unnecessary suffering of animals used in these tests.

3. A new LD_{50} test on the mixture, when there is apparently an individual oral LD_{50} reported (600 mg/kg in mice; see number 2 above) for the o-ethylphenol isomer, is absolutely unwarranted. Furthermore, there are two EPA reports cited above that also apparently contain acute toxicity data on m- and 4-ethylphenol, respectively. These should be assessed prior to proposing any new lethal dose testing. Any new information gathered from this test will not enhance the understanding of the acute oral toxicity of the mixture nor is acute toxicity needed for the individual isomers, as apparently at least one of the three has been tested (and this is sufficient for the category as a whole using the "bridging-of-data" approach). Merisol has stated in its own test plan that mixtures of cresols do not produce toxic interactions among the isomers and a similar pattern would be expected for the mixture of ethylphenols (we agree with this conclusion). In summary, acute oral toxicity testing is completely unwarranted and a violation of the HPV program principles. If for some reason, Merisol insists on conducting an acute oral toxicity test for the mixture of ethylphenols mixture, we urge the company to use the *in-vitro* cytotoxicity assays. This approach was incorporated into the HPV program as a result of the National Toxicology Program- and National Institute of Environmental Health Sciences-sponsored *Workshop on International on* In-Vitro *Methods*, held on October 17-20, 2000. This workshop reviewed the validation status of available *in-vitro* methods for predicting acute oral toxicity (among other goals). As a result of this workshop, the EPA encouraged those participating in the HPV program to "consider using the recommended *in-vitro* tests…as a supplemental component in conducting any new *in-vivo* acute oral toxicity studies…[and] to note the intention to use these protocols in the HPV Challenge test plans submitted to EPA." The two *in-vitro* tests recommended are the neutral red uptake assays using the mouse fibroblast cell line BALB/c 3T3 and normal human keratinocytes. Guidance on these recommended *in-vitro* tests, protocols for their use and a reporting template for results can be found on the ICCVAM Web site at *http://iccvam.niehs.nih.gov/docs/docs.htm#invitro*. Finally, although Merisol states that it "may" use alternative testing strategies, it should be noted that its proposal to use the traditional LD_{50} is unacceptable under any circumstances as TG 401 is being deleted internationally.

4. Merisol proposes conducting an Ames *in-vitro* bacterial mutation assay (OECD No. 417), as well as a mammalian *in-vivo* erythrocyte micronucleus test (OECD No. 474). The latter test should be deleted. If the results of the Ames assay are negative, no additional *in-vivo* testing should be conducted, especially in a screening level program. The December 2000 *Federal Register* notice states that

genotoxicity testing should be conducted *in vitro* unless physical properties preclude use of such studies.

5. The same principle apples to fish toxicity, as there is an extensive Canadian report on the aquatic toxicity of the phenols, including fathead minnow LC_{50} data for 4-ethylphenol (see number 2 above). Additional studies in fish are not warranted, as the above report is an official Canadian document, the contents of which should meet the HPV SIDS requirement.

6. Finally, there is not a data vacuum surrounding isomers of ethylphenols' reproductive and developmental toxicity. A developmental toxicity study under Good Laboratory Practice's has been conducted on 2,6-xylenol as well as extensive testing on cresols, the toxicity database of which was used in part to justify the mixed ethylphenols category. The cresols are methylphenols and 2,6-xylenolol is a di-methyl phenol instead of being ethylphenols, which are the subject of this test plan. Thus, with everything that is known about the mixed xylenols category and cresols category, further testing of the ethylphenols for reproductive/developmental toxicity is unlikely to provide any new insight into this toxicity for this endpoint. Rather, an *in-vivo* study using 750 animals in stressful experiments is neither warranted nor justified. As an alternative to *in-vivo* testing, an *in-vitro* embryotoxicity test would be adequate to characterize any possible adverse reproductive effects of these materials. If, in fact, Merisol insists on further exploration of developmental endpoints, we urge it to consider the use of an *in-vitro* test for embryotoxicity (a critical endpoint in developmental toxicity) using the rodent Embryonic Stem Cell Test (EST) protocol that has been validated by the European Centre for the Validation of Alternative Methods (ECVAM). For additional information, please refer to E. Genschow et. al., "The ECVAM international validation study on *in-vitro* embryotoxicity tests: results of the definitive phase and evaluation of prediction models" (*Alternatives to Laboratory Animals* 30:151-76, 2002). If a positive result is found, the substance should be treated as a developmental toxicant/teratogen, and no further testing should be conducted under the screening-level HPV program.

7. Although some of the data identified in objection 2 above may have been generated by other companies, we strongly encourage Merisol to coordinate any new SIDS work with others who may have already conducted duplicative testing in animals. This approach is consistent with the EPA's stated goals of maximizing the use of existing data in order to limit additional animal testing. We have encouraged the EPA in past test plan comments to ensure inter-industry cooperation in the development of chemical categories and test plans, including comments on the American Petroleum Institute Petroleum Coke test plan, the Phosphite Producers HPV Consortium test plan on tris(nonylphenol)phosphite, and the General Electric test plan on p-cumylphenol. We are concerned that the EPA is not adequately encouraging inter-company and inter-industry cooperation in the development of test plans and chemical categories, thus greatly increasing the number of animals killed in the HPV program.

Summary:

Merisol has inappropriately stated that existing data available on the individual isomers of the ethylphenols will not be used to evaluate data gaps for the HPV SIDS battery. Rather, it has ignored these data, as well as other data cited in these comments, and proposed a complete SIDS battery on the mixture of these three isomers. There may be sufficient data on the individual ethylphenol isomers (which have not been included in Merisol's assessment), in conjunction with what is known from testing on methylphenols (cresols category) and di-methylphenols (mixed xylenols category), with regards to mixture interactions (or lack thereof), which would render any new animal tests with ethylphenols completely unnecessary. In spite of this fact, Merisol has proposed tests on animals and has failed to fully utilize the available toxicity data on ethylphenols to meet HPV SIDS requirements. Furthermore, conducting these new tests clearly violates Sections 1 and 8 of the animal protection agreement and the EPA's December 2000 *Federal Register* notice that states a) "In analyzing the adequacy of data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there are sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested" and b) "As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant." Conducting a new LD_{50} study and a new repeat dose/reproductive/developmental screening study on the mixture of ethylphenols without a full evaluation of the existing data available on the individual isomers violates the standard set forth in the *Federal Register* as well as good science and thoughtful toxicology.

I look forward to a prompt and favorable response to our concerns. I may be reached at 202-686-2210, ext. 302, or via email at *csandusky@pcrm.org*.

Sincerely,

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Senior Toxicologist